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(54) Optical detection of specific molecules

(57) An optical method for detecting the presence and/or behaviour of a first form of specific molecules in various substances comprises the steps of applying a sample 6 of the substance (e.g. blood) to a molecular adsorbed layer 4 formed on an appropriate boundary surface of light transmitting device 2. Layer 4 embodies a second form of specific molecules (e.g. antibodies) capable of attracting specific molecules (e.g. antigens) from the sample for chemical combination therewith. Light is injected into the device so that at least a part thereof enters layer 4. The light output from the device is then detected for assessment of the effect thereon of any molecules of the first form absorbed into layer 4. Polarizers 9 & 10 are used because the orthogonal light components are attenuated differently according to changes in anisotrophy. Instead of the planar waveguide of Fig. 1 a triangular prism with an adsorbed layer may be used.

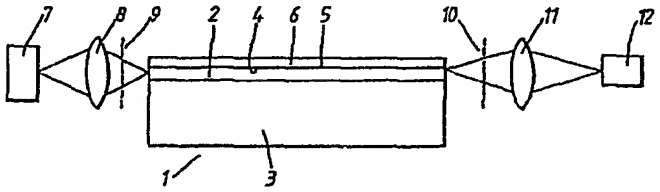

FIG. 1.

GB 2 156 970 A

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The drawings originally filed were informal and the print here reproduced is taken from a later filed formal copy.

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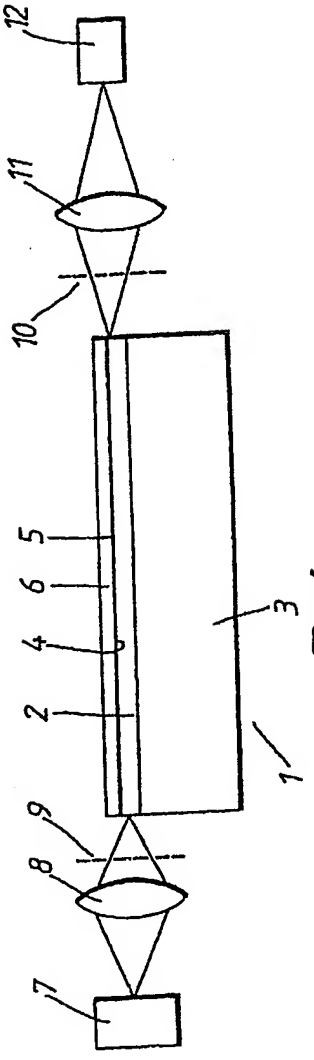

FIG.1.

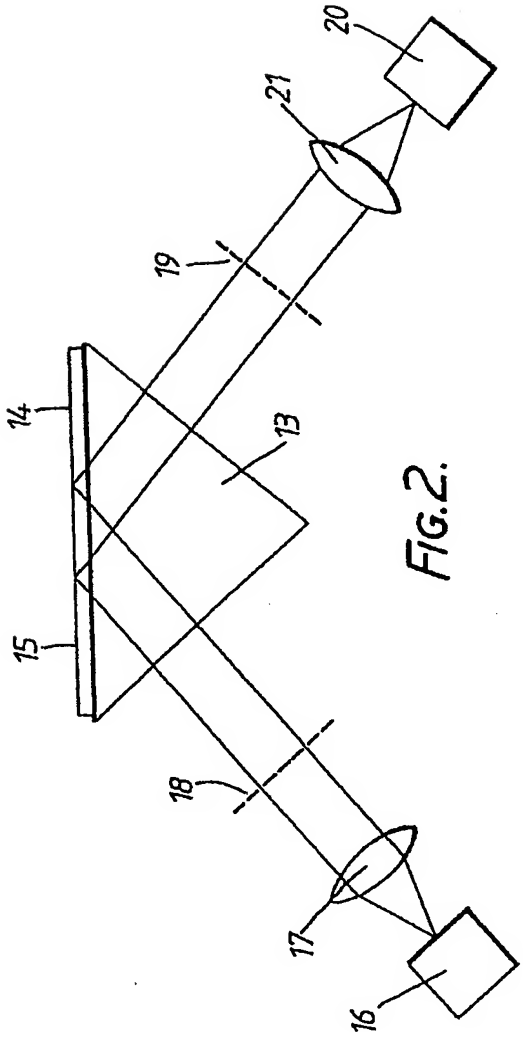

FIG.2.

SPECIFICATION

Improvements relating to optical detection methods and apparatus

This invention relates to methods and apparatus for detecting and/or monitoring or quantifying the presence and/or behaviour of certain specific molecules in various substances and the invention is especially, but not exclusively, applicable to the clinical detection of antigens in blood samples and to the monitoring of clincial diagnostic reactions involving enzymes, for example.

It is already known to detect the presence of antigens in blood samples by causing the antigens to be attracted into an adsorbed layer of a substance which contains antibodies and which constitutes the gate electrode of an insulated-gate field-effect transistor (IGFET) so that the current flow between the source and drain of the transistor is varied in accordance with the presence of antigens absorbed into the gate electrode. The transistor current flow is monitored to detect the presence of antigens after which the transistor will be disposed of.

The present invention accordingly has in view a detecting and/or monitoring or quantifying method which makes use of a significantly cheaper disposable device than the IGFET referred to above and which enables inter alia the take-up of antigens by the absorbed layer in the antigen detection application of the invention to be monitored over a protracted period (e.g. 15 minutes).

In accordance with the present invention there is provided an optical method for detecting and/or monitoring or quantifying the presence and/or behaviour of a first form of specific molecules in various substances, which method comprises the steps of applying a sample of one of said substances to a molecular adsorbed layer which is formed on an appropriate boundary surface of a relatively cheap light transmitting device and which embodies a second form of specific molecules capable of attracting specific molecules of the first form to said adsorbed layer for chemical combination therewith, injecting light into said device so that at least a part thereof enters the adsorbed layer and detecting, monitoring or measuring the light output from said device for assessment of the effect thereon of any molecules of the second form which have been absorbed unto the adsorbed layer.

In carrying out the present invention the light transmitting device may comprise a disposable planar optical waveguide with the adsorbed layer being provided on one boundary surface of the waveguide, or alternatively, the device may comprise a simple cheap prism (e.g. triangular) having the adsorbed layer provided on one face thereof. The disposable planar optical waveguide may simply comprise a glass slide of the form commonly used in microscopy provided with a surface layer of different refractive index.

When a planar optical waveguide is used, light injected into one end of the waveguide will be propagated through the waveguide so that evanescent waves of the guided light will penetrate into the adsorbed layer of the device where they will be absorbed and/or otherwise modified (e.g. velocity differential between components) by the material of the layer and to a degree dependent upon the presence of specific moledules of the first form absorbed in the adsorbed layer and thereby producing attenuation or a change in attenuation of the guided light-wave which can be detected and/or measured.

The material of the adsorbed layer may also be anisotropic in which case the propagation characteristics of orthogonal polarised components of the light (e.g. magnetic and electric) injected into the waveguide will be influenced by the anisotropy of the layer so that the electrical and magnetic mode propagation constants will differ as a function of the anisotropy and the degree of absorption of each mode polarisation by the adsorbed layer will usually be different. Consequently, changes in anisotropy of the adsorbed layer due to the absorption therein of specific molecules of the first form will affect the attenuation of the orthogonal polarised components of light injected into the waveguide and thus the measured intensities of these polarisations may be used to provide an indication of any absorption of specific molecules of the first form into the adsorbed layer.

Similarly, in the alternative case where an optical prism is utilised orthogonal polarised light components may be injected into the prism but in this case it may be arranged that the light beam itself passes through the adsorbed layer and after internal reflection from the outer surface of the applied substance embodying the specific molecules of the first form it passes back through the adsorbed layer and thus the intensities of the two polarisations of light may be detected and/or measured for determining whether and in what quantity molecules of the first form have been absorbed into the adsorbed layer.

As alternatives to the above-described techniques of effectively detecting and/or measuring the absorption or changes in the absorption of light at the propagation wavelength (usually ultraviolet spectral range) by the adsorbed layer it is also envisaged that changes in birefringence or Raman back-scattering of light in the adsorbed layer may be utilised to detect the absorption of specific molecules into the adsorbed layer. These alternative techniques enable a wider range of light wavelengths to be used.

It is contemplated that the method of the present invention and the apparatus for carrying it out will have many applications in the chemical and medical analytical and diagnostic fields but two especially envisaged applications are in the detection and/or monitoring of antigens in blood samples and in monitoring clinical diagnostic reactions involving enzymes.

By way of example the present invention will now be described with reference to the accompanying drawing in which;

Figure 1 shows a schematic diagram of an optical waveguide apparatus for detecting and/or

measuring the absorption of specific molecules from a blood sample into an adsorbed layer of the waveguide; and,

Figure 2 shows a schematic diagram of an optical prism apparatus for detecting and/or measuring the absorption of specific molecules of a blood sample into an adsorbed layer of the prism.

Referring to Figure 1 of the drawing the apparatus depicted comprises a planar optical dielectric waveguide 1 conveniently consisting of a thin glass film 2 of one refractive index supported on a glass substrate of a different refractive index, or the film 2 may be surface layer of gradient refractive index supported on a susbtrate of uniform refractive index. The thin- film 2 of the waveguide has applied to it an adsorbed surface layer 4 of a material which in the present example contains specific antibodies. In the adsorption process these antibodies align with a distinct and well-defined orientation with respect to the waveguide surface 5 and their function is to attract any antigens from a blood sample 6 applied to the waveguide surface 5. The chemical combination of antibodies in the adsorbed layer 4 and antigens in the applied blood sample 6 occurs at a well-defined molecular position maintaining the orientation of the adsorbed layer 4 and in practice the quantity of antibodies will be sufficient to maintain the depth of the adsorbed layer 4, with or without antigens, within or nearly equal to the penetration depth of the transverse evanescent field of the propagated light into the adsorbed layer.

Light (ultra-violet) derived from a light source 7 is focused by a convex lens 8 on to one end of the waveguide film 2 after passing it through a polariser 9 for the generation of polarised light. This polarised light is propagated along the waveguide with the transverse evanescent field produced by the guided light penetrating into the adsorbed layer 4. The degree of absorption and/or modification of the guided light by the adsorbed layer 4 will depend upon the chemical combination of antigens from the blood sample 6 with antibodies in the adsorbed layer 4. This dependence may result from changes in the anisotropy of the material of the adsorbed layer 4 due to the presence of antigens. Moreover, the orthogonal polarised light components (electric and magnetic) of the guided light will be attenuated differentaly according to these changes in anisotropy. The orthogonal polarised light components emerging from the other end of the waveguide are applied to a polariser 10 which is arranged at 45° to the orthogonal polarisation (electric and magnetic) directions so that the change in output from the polariser 10 which is focused by a convex lens 11 on to an optical detector 12 corresponds to the difference between propagation constants of the polarised components. The polarised output is thus dependent upon the changes in the absorption of light by the adsorbed layer due to the presence of antigens attracted to the layer by the antibodies therein.

These changes in absorption can all be detected and/or monitored or quantified sufficiently fast in time allowing observation of the take-up of antigens by antibodies. Since the transverse evanscent field does not penetrate beyond the adsorbed layer 2 the background optical characteristics presented by the blood sample itself do not influence the detection of antigens and moreover the method only requires a very small volume of blood sufficient to provide a layer a few microns thick and spread over a few square centimetres of waveguide surface area.

Referring now to Figure 2, this shows an alternative form of apparatus including a triangular prism 13 which has an adsorbed layer 14 embodying antibodies and corresponding to layer 4 of the Figure 1 apparatus. The blood sample containing antigens is applied at 15 to the layer 14. Light (ultra-violet) from a light source 16 after collimation by a convex lens 17 and passing through a polariser 18 enters the prism 13 and after passing through the adsorbed layer 14 is internally reflected from the upper surface of liquid blood sample 15. The light will be attenuated inter alia by the antigens attracted into the adsorbed layer by the antibodies therein. The attenuated light emerging from the prism 13 passes through a polariser 19 before it is focussed on to an optical detector 20 by a convex lens 21. The detector output and/or indication affords an indication of the presence or absence of antigens in the blood sample applied to the prism.

CLAIMS

1. An optical method for detecting and/or monitoring or quantifying the presence and/or behaviour of a first form of specific molecules in various substances, said method comprising the steps of applying a sample of one of said substances to a molecular adsorbed layer which is formed on an appropriate boundary surface of a relatively cheap light transmitting device and which embodies a second form of specific molecules capable of attracting specific molecules of the first form to said adsorbed layer for chemical combination therewith, injecting light into said device so that at least a part thereof enters the adsorbed layer and detecting, monitoring or measuring the light output from said device or otherwise assessing the effect thereon of any molecules of the second form which have been absorbed into the adsorbed layer.

2. An optical method as claimed in claim 1, in which the light transmitting device comprises a disposable planar optical waveguide with the adsorbed layer being provided on one boundary surface of the waveguide and in which light is injected into one end of the waveguide so that it is propagated through the waveguide whereby evanescent waves of the guided light will penetrate into the adsorbed layer of the device where they will be absorbed and/or otherwise modified by the material of the layer to a degree dependent upon the presence of specific molecules of the first form absorbed in the adsorbed layer and thereby producing attenuation or a change in attenuation of the guided light-wave which can be detected and/or measured.

3. An optical method as claimed in claim 1, in

which the light transmitting device comprises a simple prism (e.g. triangular) having the adsorbed layer provided on one face thereof.

4. An optical method as claimed in claim 2, in which the disposable planar optical waveguide comprises a glass slide with a surface layer of different refractive index.

5. An optical method as claimed in claim 2, in which the adsorbed layer is anisotropic whereby the propagation characteristics of orthogonal polarised components of the light injected into the waveguide will be influenced by the anisotropy of the layer so that the electrical and magnetic mode propagation constants will differ as a function of the anisotropy and the degree of absorption of each mode polarisation will be different.

6. An optical method as claimed in claim 3, in which orthogonal polarised light components are injected into the prism and in which the light beam is arranged to pass through the adsorbed layer and after internal reflection from the outer surface of the applied substance embodying the specific molecules of the first form to pass back through the adsorbed layer, the intensities of the two polarisations of light being detected and/or measured for determining whether and in what quantity molecules of the first form have been absorbed into the adsorbed layer.

7. An optical method as claimed in claim 1, in which changes in birefringence or Raman backscattering of light in the adsorbed layer are utilised to detect the absorption of specific molecules into the adsorbed layer.

8. The optical detecting and/or measuring method hereinbefore described with reference to Figure 1 or Figure 2 of the accompanying drawing.

9. The optical waveguide apparatus suitable for detecting and/or measuring the absorption of specific molecules from a blood sample into an adsorbed layer of the waveguide substantially as hereinbefore described with reference to Figure 1 of the accompanying drawing.

10. The optical prism apparatus suitable for detecting and/or measuring the absorption of specific molecules of a blood sample into an adsorbed layer of the prism substantially as hereinbefore described with reference to Figure 2 of the accompanying drawing.

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